We have previously described a signaling center in birds, the Frontonasal Ectodermal Zone (FEZ) that regulates growth and patterning of the frontonasal process (FNP). The FEZ is comprised of ectoderm flanking a boundary between *Sonic hedgehog* (*Shh*) and *Fibroblast growth factor 8* (*Fgf8*) expression domains within the FNP ectoderm. Mechanisms that govern formation of the FEZ are unknown, but the expression patterns of *Bmps* and their receptors suggest that BMP signaling could play a role. Our objective was to assess the role of BMPs during FEZ formation. We blocked BMP signaling in the FNP prior to FEZ formation by injecting a replication competent avian retrovirus encoding the BMP antagonist *Noggin* into the mesenchyme beside the forebrain at HH stage 10. We assessed FEZ formation 72 h after infection (~βHH22) and observed that *Shh* expression was reduced or absent and *Fgf8* expression was not affected. We also observed changes in gene expression in neural crest cells. *Bmp2* expression was absent and *Bmp4* expression was expanded proximally. Embryos developing for 72 and 96 h after infection exhibited malformations of the FNP that indicated the FEZ did not form. Electroporating a constitutively active BMP receptor into the ectoderm 24 h after infection (~βHH14) partially rescued this phenotype. These data indicate that BMP signaling mediates interactions between the neural crest mesenchyme and the ectoderm of the FNP to regulate FEZ formation. We are currently assessing the signaling and patterning properties of the FEZ that forms after blocking BMP signaling in the FNP.

doi:10.1016/j.ydbio.2007.03.605

Program/Abstract # 305
Bmp signalling in the epibranchial placodes
Nadja N. Kriebitz, Anthony Graham, Esther Bell
MRC Centre for Developmental Neurobiology, KCL, London, UK

A number of lines of evidence have highlighted a role for bone morphogenetic proteins (BMPs) in the formation of the epibranchial placodes. It has been shown that the pharyngeal endoderm induces the formation of the epibranchial placodes via the action of BMP7. More recently, we have found that Bmp4 is expressed in the placodes themselves. We have also analyzed the expression profiles of members of the Cerberus/Dan family of BMP antagonists, and we find that the location and timing of PRDC expression within the endoderm of the arches coincides with epibranchial placode formation. These observations strengthen the idea that BMP signaling has an important and ongoing function in the formation of the epibranchial placodes. It is likely that BMP7 has a role in inducing the placodes and BMP4 a role in maintaining them, and that the activity of these two factors is modulated by PRDC. We are currently investigating the precise roles of BMP signaling, and the importance of the levels of BMP activity, for placode development using both gain of function and loss of function approaches in the chick embryo.

doi:10.1016/j.ydbio.2007.03.606

Program/Abstract # 306
Study of Xenopus orthologs of novel genes expressed in the mouse AVE
Ana C. Silva [1], Marta Vitorino [2], Mario Filipe [1], Sara Marques [2], Jörg D. Becker [3], Herbert Steinbeisser [4], A. Belo [2]
[1] *Dev. Biol. Unit, IGC, Oeiras, Portugal*
[2] *CBME, Univ. Algarve, Faro, Portugal*
[3] *Affymetrix Core Unit, IGC, Oeiras, Portugal*
[4] *Human Genet Institute, Heidelberg Univ., Heidelberg, Germany*

The classical transplantation experiments of Spemann and Mangold and the molecular characterization of Spemann organizer have established that the amphibian organizer could be subdivided into head and trunk inducing regions. Organizer genes also exhibit different patterning properties. Cerberus, dickkopf1 or frzb can induce anterior neural structures, whereas chordin or noggin can only induce more caudal structures. In the peri-gastrulation mouse embryo, the orthologs of the organizer genes are found mainly in the anterior end of the primitive streak, its derived axial mesendoderm and in the extra-embryonic anterior visceral endoderm (AVE). Together with the orthologs of secreted head inducers of the organizer, several transcription factors with known roles in forebrain specification are also expressed in the AVE before gastrulation. Hence, the AVE was proposed to be the mouse head-organizer. In order to further characterize the molecular mechanisms involved in the early forebrain induction, we have carried out a screen for genes differentially expressed in the AVE. Interestingly most of the *Xenopus* orthologs of novel genes expressed in AVE were detected in the dorsoanterior endoderm, a region considered to be the topological equivalent of the AVE. The combined results of the studies conducted in these two species will further contribute to the understanding of how the vertebrate head is induced and patterned.

doi:10.1016/j.ydbio.2007.03.607

Program/Abstract # 307
Autonomous sorting & surface segregation of primitive endoderm in mouse embryoid bodies
Robert Moore, Malgorzata E. Rula, Kathy Q. Cai, Dong-Hua Yang, Cory M. Staub, Callinice D. Capo-chichi, R. Smith, Xiang-Xi Xu
Dept. of Med. Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA

The genesis of the primitive endoderm can be mimicked in vitro by the aggregation of either dissociated embryonic stem cells or F9 embryonal carcinoma cells in suspension to form embryoid bodies (EBs). We present morphological evidence that nascent primitive endoderm cells are first born within the interior of EBs and translocate concomitantly to the surface exterior. Furthermore, we have generated heterotypic EBs by mixing undifferentiated F9 cells and F9 cells differentiated into endoderm by prior exposure to retinoic acid. Within composite EBs the

differentiated endoderm cells sorted circumferentially while the undifferentiated F9 cells remained predominantly internal. This indicates that the acquisition of a surface position is an intrinsic property of endoderm epithelia. Disabled-2 (Dab2), an endocytic adaptor protein that mediates the directional transport of clathrin-coated cargos, is required for the spontaneous surface sorting and positioning of the endoderm cells. When Dab2 expression was compromised, the differentiated F9 cells no longer localized correctly and were distributed throughout the interior of the EBs. These results support a model where primitive endoderm cells are first formed within the interior of the inner cell mass of the preimplantation mouse blastocyst and are subsequently sorted to the surface by a Dab2-dependent mechanism. We propose that the autonomous property of epithelial cells to generate polarity is the factor responsible for surface positioning of epithelia.

doi:10.1016/j.ydbio.2007.03.608

Program/Abstract # 308

Thyrotropin-releasing hormone precursor—A novel marker of the mouse definitive endoderm

Kristen D. McKnight [1], Pamela A. Hoodless [2]
[1] *Genetics Graduate Program, University of British Columbia, Vancouver, BC, Canada*
[2] *Department of Medical Genetics, University of British Columbia, BC, Canada*
[3] *Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC, Canada*

Gastrulation is one of the most critical events of embryogenesis, generating the three primary germ layers (endoderm, mesoderm and ectoderm) that will give rise to the tissues of the developing embryo. Of the germ layers the least is known about the definitive endoderm (DE), which gives rise to the lungs, digestive tract, liver and pancreas. This is due in large part to the lack of genetic markers specific for the DE as many of the current markers, including *Cerl*, *Foxa2* and *Sox17*, are also expressed in the visceral endoderm (VE), an extraembryonic tissue. Using Affymetrix GeneChips and Serial Analysis of Gene Expression (SAGE) we have identified a novel marker of the mouse DE—*Thyrotropin-releasing hormone precursor* (*Trh*). We have characterized the expression of *Trh* throughout mouse gastrulation and early organogenesis stages using whole mount *in situ* hybridization. Our expression data shows that *Trh* is expressed in newly formed DE cells and is subsequently expressed in the entire DE before becoming downregulated as the DE is patterned. The dynamic expression pattern of *Trh* is in accordance with recent fate mapping experiments detailing the movement of the DE during gastrulation. Preliminary experiments suggest that *Trh* is absent from the VE, being expressed in a mutually exclusive pattern with *Pem* (a marker of the extraembryonic visceral endoderm). These results point to *Trh* being an exclusive DE marker.

doi:10.1016/j.ydbio.2007.03.609

Program/Abstract # 309

FoxD3 regulation of mesoderm induction in the zebrafish embryo

Lisa L. Chang, Daniel S. Kessler
Dept. of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

Nodal ligands are required for germ layer induction in vertebrates. During zebrafish gastrulation, the expression domain of the Nodal-related genes, Cyclops (Cyc) and Squint (Sqt), overlaps that of FoxD3 in the shield, suggesting a possible role for FoxD3 in mesoderm development. Overexpression of FoxD3 results in expansion of Cyc expression and dorsal mesoderm markers. Knockdown results in reduced expression of these markers and 24-h embryos show a phenotype similar to Nodal pathway mutants. To determine the functional interaction of FoxD3 with the Nodal pathway we examined Antivin-overexpressing and MZoep mutant embryos. FoxD3 does not rescue or induce ectopic mesoderm indicating that FoxD3 is dependent on a functional Nodal pathway for dorsal mesoderm induction. A FoxD3 mutant, Sym1, where the mutation inactivates the FoxD3 gene has been reported. The phenotype shows craniofacial defects and delayed/reduced development of chromatophores. From our results and our model for FoxD3 activity we predict early gastrulation deficiencies and defects in tissues derived from dorsal mesoderm. Our preliminary results indicate that the sym1 protein retains partial function as its overexpression induces Cyc and dorsal mesoderm markers. Future work will examine sym1 embryos for unappreciated defects in mesodermal gene expression and axial development. Results suggest that dorsal mesoderm induction is regulated, at least in part, by FoxD3. We hypothesize that FoxD3 regulates Nodal expression in the zebrafish shield by repressing a negative regulator of Nodal expression, thus indirectly promoting Nodal expression and mesoderm development.

doi:10.1016/j.ydbio.2007.03.610

Program/Abstract # 310

Genetic analysis of Fgf gene function in the limb

Francesca Mariani [1], Christina Ahn [1], David Ornitz [2], Gail Martin [1]
[1] *Dept. of Anatomy, University of California, San Francisco, CA*
[2] *Dept. of Molecular Biology and Pharmacology, Washington University, St. Louis, MO*

The tetrapod limb emerges from the flank of the embryo as a bud of mesenchyme encased in an epithelial hull. Substantial growth and differentiation of this bud gives rise to a scaffold of skeletal elements that can vary among species in their size, number and shape. At the distal edge of the bud the apical ectodermal ridge (AER), produces signals essential for limb development. In the mouse, four *Fgf* genes, *Fgf4*, *Fgf8*, *Fgf9* and *Fgf17*, are expressed specifically in the AER and may be the critical genes that provide these essential signals. Using a genetic